COMPARATIVE TOXICITY STUDY OF TETRACHLOROBENZYLTOLUENES (UGILEC 141) AND POLYCHLOROBIPHENYLS (AROCLOR 1254 AND PCB-77) IN AH-RESPONSIVE (C57BL/6) AND AH-NONRESPONSIVE (DBA/2) MICE.

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ABSTRACT:

Similar toxicological alterations were induced by the technical tetrachlorobenzyltoluene-mixture, Ugilec 141 (used as an alternative for PCBs in hydraulic fluids), Aroclor 1254 and PCB-77 in Ah-responsive and Ah-nonresponsive mouse strains.

INTRODUCTION

Tetrachlorobenzyltoluenes (TCBTs), under the tradename Ugilec 141, are used as alternatives for polychlorobiphenyls (PCBs) in hydraulic systems since 1984, especially by the German mining industry. Since 1985 evidence has been obtained for contamination of water and sediment with Ugilec 141 in the rivers Rur and Lippe that drain the German coalmining area in Nordrhein-Westphalen [1]. Accumulation of Ugilec 141 in fish from these rivers was observed to levels of 15 and 25 mg/kg product in the German mining area [2,3], while levels up to 4.8 mg/kg were observed in red eel of the river Roer at the Dutch/German border [4].

The remarkable structural resemblance between TCBTs and PCBs prompted us to perform a comparative toxicity study in Ah-responsive, C57BL/6 mice and Ah-nonresponsive, DBA/2 mice with special emphasis on characteristic PCB-induced effects, such as hepatomegaly, induction of cytochrome P450IA1, and reduction of hepatic and plasma retinoids and plasma thyroxin levels [5,6].

MATERIALS AND METHODS

Female mice (6 per group), 8-10 weeks old, were exposed to a single, intraperitoneal dose of 50 and 200 mg/kg Ugilec 141 (Promochem, Wesel, W-Germany), of 50 and 200 mg/kg Aroclor 1254 (Analabs Inc. No. Haven, Conn. USA) and of 50 mg/kg 3,4,3',4'-tetrachlorobiphenyl (PCB-77, Chrompack, Middelburg, The Netherlands), or cornoil (5 ml/kg), which was used as a vehicle. Blood was collected at day 1, 4 and 7 after exposure by tailbleeding under ether anaesthesia. At day 7 (termination time) blood, liver and thyroid gland were collected for biochemical analyses and histopathological examination. Liverweight and bodyweight gain were recorded.

Analysis of hepatic and plasma vitamin A was performed according to previously published methods [6]. Plasma thyroid hormone analyses were performed by chemoluminescence immunoassay, using commercially available kits (Amerlite TT4 assay, Amersham Internat. plc., Amersham, UK). Preparation of hepatic microsomes, total cytochrome P450 measurements [7], EROD and PROD assays [8] were performed according to previously published methods. Histopathological examination was performed on formaline-fixed slides, stained with Haematoxilin/Eosin according to standard procedures.

RESULTS

Exposure of Ah-responsive, C57BL/6 mice to either Ugilec 141, Aroclor 1254 or PCB-77 resulted in a significant increase in liverweight by respectively, 141%, 133% and 118% of controls (Table 1). No significant increase in liverweight was observed in the Ah-nonresponsive DBA/2 strain. The increased liverweight in C57BL/6 mice was accompanied by histological alterations, such as mild centrilobular megalocytosis and occasionally necrosis, especially in the Ugilec 141 (200 mg/kg) treatment group (data not shown).

In addition to hepatomegaly, a significant induction of hepatic cytochrome P450 and ethoxyresorufin-o-deethylase (EROD) activity was observed in C57BL/6 mice following exposure to either Ugilec 141, Aroclor 1254, or PCB-77 (Table 1). In DBA/2 mice hepatic cytochrome P450 content and EROD activity were also increased by Ugilec 141, Aroclor 1254 and PCB-77 but to a much lesser extent as in the C57BL/6 mice. However, pentoxyresorufin-o-deethylase (PROD) activity was significantly induced in C57BL/6 mice by Aroclor 1254 and PCB-77 and in

DBA/2 mice by Aroclor 1254 only (Table 1).

In tandem reductions in plasma total thyroxin (TT4) and plasma retinol concentrations were observed in both strains of mice, following exposure to either Ugilec 141, Aroclor 1254, or PCB-77.

Table 1

Effects of UGILEC 141, AROCLOR 1254 and PCB-77 on some selected toxicity parameters in Ah-responsive (C57BL/6) and Ah-nonresponsive (DBA/2) mice

Mousestrain	Parameter	Relative effect (% of control)		
		UGILEC 141	AROCLOR 1254	PCB-77
C57BL/6	liverweight[1]	141 ± 15	133 ± 5	118 ± 14
	cyt. P450[1]	165 ± 21	266 ± 96	165 ± 94
	EROD activity[1]	252 ±237	850 ±417	3500 ±1750
	PROD activity[1]	ns	1680 ±836	609 ±273
	plasma TT4[1]	55 ± 5	48 ± 13	66 ± 14
	plasma retinol[2]	78 ± 11	74 ± 12	56 ± 13
DBA/2	PROD activity[1]	ns	345 ±161	ns
	plasma TT4[3]	56 ± 16	50 ± 11	61 ± 20
	plasma retinol[3]	56 ± 13	41 ± 22	33 ± 10

Note: All data presented in this table are statistically significant different from controls (Students t'-test $P<0.05$) and are obtained from the highest dose treatment groups, e.g., 200 mg/kg for Ugilec 141 and Aroclor 1254 and 50 mg/kg for PCB-77. 1: data obtained at day 7; 2: data from day 1 ; 3: data from day 4 of exposure; ns: statistically not significant.

Significant reductions of TT4 concentrations (Table 1) were observed in C57BL/6 and DBA/2 mice that were already apparent at 24 hours after exposure. Histological examination revealed mild hypertrophy of the thyroid follicle cells, especially following Ugilec 141 (200 mg/kg) treatment. Plasma retinol reductions were at the lowest levels at day 1 in C57BL/6 mice and at day 4 in DBA/2 mice (Table 1). Hepatic retinoid concentrations were not altered in the DBA/2 mice, while in the C57BL/6 mouse the hepatic retinol content was reduced by Ugilec 141, Aroclor 1254 and PCB-77 to resp. 66%, 43% and 85% of cornoil-treated controls.

DISCUSSION

The technical TCBT-mixture, Ugilec 141 caused a similar spectrum of toxicological alterations as the technical PCB mixture, Aroclor 1254

and the model toxic PCB congener 3,3',4,4'-tetrachlorobiphenyl (PCB-77). The Ah-mediated biochemical changes, such as induction of cytochrome P450IA1 and accompanying EROD induction [5], and hepatomegaly were induced by both TCBTs and PCBs. The EROD induction potency of Ugilec 141 however, was considerably less than that of PCB-77 and Aroclor 1254. This may result from the fact that TCBTs deviate to a larger extend from a planar configuration (an essential structural requirement for EROD-induction) than Aroclor 1254 and PCB-77, in particular. Interestingly, induction of EROD, cytochrome P450 and hepatomegaly by Ugilec 141 also segregated with Ah-responsiveness in the mousestrains investigated. This indicates that Ugilec 141 exerts, at least part of its toxic effects through a similar mechanism as PCBs and other halogenated aromatic hydrocarbons [5]. Moreover this suggests that Ugilec 141 may also induce other Ah-mediated pathological alterations not investigated in this study, such as thymic atrophy and teratogenicity.

In addition, typical non-Ah mediated biochemical responses of PCBs, such as plasma retinol and thyroxin reduction [6] were induced by Ugilec 141 in both mouse-strains. This indicates that exposure of mice to Ugilec 141 may result in the production of metabolites with a high binding affinity to transthyretin and accompanying distortion of plasma thyroxin and retinol transport similar to PCB-77 [6] and Aroclor 1254 [9].

In conclusion, no apparent differences were observed in toxicity between Ugilec 141 and the PCBs, Aroclor 1254 and PCB-77, which turns-Ugilec 141 and related TCBTs into very bad alternatives for PCBs.

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